

C2949-PCT.txt
SEQUENCE LISTING

<110> Collen Research Foundation VZW
Jacquemin, Marc
Saint-Remy, Jean-Marie

<120> Variable antibodies

<130> C2949-PCT

<150> GB 0319118.6

<151> 2003-08-14

<150> GB 0319345.5

<151> 2003-08-18

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<170> PatentIn version 3.1

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<223> KRIX-1 heavy chain variable region

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<222> (1)..(57)

<223> nucleotide sequence encoding the leader sequence

C2949-PCT.txt

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<223> nucleotide sequence encoding CDR1

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<222> (235)..(285)

<223> nucleotide sequence encoding CDR2

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<222> (382)..(435)

<223> nucleotide sequence encoding CDR3

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Met Asp Trp Thr Trp Arg Ile Leu Phe Leu Val Ala Ala Ala Thr Gly
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gcc cac tcc cag gtg caa ctg gtg caa tct ggg gct gag gtg aag aag      96
Ala His Ser Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys
20     25     30

cct ggg gcc tca gtg aag gtc tcc tgc aag acc tct gga tac aac ttc      144
Pro Gly Ala Ser Val Lys Val Ser Cys Lys Thr Ser Gly Tyr Asn Phe
35     40     45

acc ggc tac tct gct tct gga cat atc ttc acc gcc tac tct gtg cac      192
Thr Gly Tyr Ser Ala Ser Gly His Ile Phe Thr Ala Tyr Ser Val His
50     55     60

tgg gtg cga cag gcc cct gga caa ggg ctt gag tgg atg gga agg atc      240
Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met Gly Arg Ile
65     70     75     80

aac cct aac agt ggt gcc aca gac tat gca cat aaa ttt cag ggc agg      288
Asn Pro Asn Ser Gly Ala Thr Asp Tyr Ala His Lys Phe Gln Gly Arg
85     90     95

gtc acc atg tcc agg gac acg tcc atc agc aca gcc tac atg gaa ctg      336
Val Thr Met Ser Arg Asp Thr Ser Ile Ser Thr Ala Tyr Met Glu Leu
100    105    110

agc agg ctg aca tct gac gac acg gcc atg tat tac tgt gcg aga gcc      384
Ser Arg Leu Thr Ser Asp Asp Thr Ala Met Tyr Tyr Cys Ala Arg Ala
115    120    125

gac aac tat ttc gat att gtg act ggc tat act tct cat tac ttt gac      432
Asp Asn Tyr Phe Asp Ile Val Thr Gly Tyr Thr Ser His Tyr Phe Asp
130    135    140

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C2949-PCT.txt

tac tgg ggc cgg gga acc ctg gtc acc gtc tcc tca gcc tcc acc aag 480
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ggc cca tcg gtc ttc c 496
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<223> nucleotide sequence encoding CDR3

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Met Asp Trp Thr Trp Arg Ile Leu Phe Leu Val Ala Ala Ala Thr Gly
 1 5 10 15

Ala His Ser Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys
 20 25 30

Pro Gly Ala Ser Val Lys Val Ser Cys Lys Thr Ser Gly Tyr Asn Phe
 35 40 45

Thr Gly Tyr Ser Ala Ser Gly His Ile Phe Thr Ala Tyr Ser Val His
 50 55 60

C2949-PCT.txt

Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met Gly Arg Ile
 65 70 75 80

Asn Pro Asn Ser Gly Ala Thr Asp Tyr Ala His Lys Phe Gln Gly Arg
 85 90 95

Val Thr Met Ser Arg Asp Thr Ser Ile Ser Thr Ala Tyr Met Glu Leu
 100 105 110

Ser Arg Leu Thr Ser Asp Asp Thr Ala Met Tyr Tyr Cys Ala Arg Ala
 115 120 125

Asp Asn Tyr Phe Asp Ile Val Thr Gly Tyr Thr Ser His Tyr Phe Asp
 130 135 140

Tyr Trp Gly Arg Gly Thr Leu Val Thr Val Ser Ser Ala Ser Thr Lys
 145 150 155 160

Gly Pro Ser Val Phe
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<222> (130)..(164)

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C2949-PCT.txt

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<223> nucleotide sequence encoding leader peptide

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1				5				10					15			

gat	acc	acc	gga	gaa	att	gtg	ttg	acg	cag	tct	cca	ggc	acc	ctg	tct	96
Asp	Thr	Thr	Gly	Glu	Ile	Val	Leu	Thr	Gln	Ser	Pro	Gly	Thr	Leu	Ser	
			20				25					30				

ttg	tct	cca	ggg	gaa	aga	gcc	acc	ctc	tcc	tgc	agg	gcc	agt	cag	agt	144
Leu	Ser	Pro	Gly	Glu	Arg	Ala	Thr	Leu	Ser	Cys	Arg	Ala	Ser	Gln	Ser	
		35				40					45					

gtt	gcc	agc	gcc	tac	tta	gcc	tgg	tac	cag	caa	aaa	cct	ggc	cag	gct	192
Val	Ala	Ser	Ala	Tyr	Leu	Ala	Trp	Tyr	Gln	Gln	Lys	Pro	Gly	Gln	Ala	
	50					55					60					

ccc	agg	ctc	ctc	atc	tat	ggg	gca	tcc	agt	agg	gcc	acc	gac	atc	cca	240
Pro	Arg	Leu	Leu	Ile	Tyr	Gly	Ala	Ser	Ser	Arg	Ala	Thr	Asp	Ile	Pro	
65				70					75						80	

cac	agg	ttc	agt	ggc	agt	ggg	tct	ggg	aca	gac	ttc	act	ctc	acc	atc	288
His	Arg	Phe	Ser	Gly	Ser	Gly	Ser	Gly	Thr	Asp	Phe	Thr	Leu	Thr	Ile	
				85				90						95		

agc	aga	ctg	gag	cct	gaa	gat	ttt	gca	gtg	tac	tac	tgt	cag	caa	tat	336
Ser	Arg	Leu	Glu	Pro	Glu	Asp	Phe	Ala	Val	Tyr	Tyr	Cys	Gln	Gln	Tyr	
			100					105					110			

ggg	acc	tca	gcc	tta	ctc	act	ttc	ggc	gga	ggg	acc	aag	gtg	gag	atc	384
Gly	Thr	Ser	Ala	Leu	Leu	Thr	Phe	Gly	Gly	Gly	Thr	Lys	Val	Glu	Ile	
		115					120					125				

aaa	cga	act	gtg	gct	gca	cca	tct	gtc	ttc	atc	ttc	ccg	cca	tct		429
Lys	Arg	Thr	Val	Ala	Ala	Pro	Ser	Val	Phe	Ile	Phe	Pro	Pro	Ser		
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<222> (211)..(231)

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<222> (328)..(357)

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<222> (1)..(60)

<223> nucleotide sequence encoding leader peptide

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Met Glu Thr Pro Ala Gln Leu Leu Phe Leu Leu Leu Leu Trp Leu Pro
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Asp Thr Thr Gly Glu Ile Val Leu Thr Gln Ser Pro Gly Thr Leu Ser
 20 25 30

Leu Ser Pro Gly Glu Arg Ala Thr Leu Ser Cys Arg Ala Ser Gln Ser
 35 40 45

Val Ala Ser Ala Tyr Leu Ala Trp Tyr Gln Gln Lys Pro Gly Gln Ala
 50 55 60

Pro Arg Leu Leu Ile Tyr Gly Ala Ser Ser Arg Ala Thr Asp Ile Pro
 65 70 75 80

His Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile
 85 90 95

Ser Arg Leu Glu Pro Glu Asp Phe Ala Val Tyr Tyr Cys Gln Gln Tyr
 100 105 110

Gly Thr Ser Ala Leu Leu Thr Phe Gly Gly Gly Thr Lys Val Glu Ile
 115 120 125

C2949-PCT.txt

Lys Arg Thr Val Ala Ala Pro Ser Val Phe Ile Phe Pro Pro Ser
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<213> Artificial Sequence

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<223> Krix-1 heavy chain forward primer

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<210> 6

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<212> DNA

<213> Artificial Sequence

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<223> Krix-1 heavy chain reverse primer

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tatggccgac gtcgactcat ttacccggag acagggagag

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<210> 7

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C2949-PCT.txt

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aaacagcctc tagactaaca ctctcccctg ttgaag

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<223> Krix-1 forward mutagenic primer Asn47Gln

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cctgcaagac ctctggatac caattcacccg gctactctgc ttctgg

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<223> Krix-1 reverse mutagenic primer Asn47Gln

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ccagaagcag agtagccggt gaattggtat ccagaggtct tgcagg

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<211> 37

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<223> Krix-1 forward mutagenic primer Thr49Ala

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<211> 37

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C2949-PCT.txt

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<223> Krix-1 reverse mutagenic primer Thr49Ala

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46

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<212> DNA

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<223> Krix-1 reverse mutagenic primer Asn47Glu

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<223> Krix-1 forward mutagenic primer Asn47Asp

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<223> Krix-1 reverse mutagenic primer Asn47Asp

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<223> scFv-KRIX-1VL reverse primer

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<211> 56

<212> DNA

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<223> scFv-KRIX-1VH forward primer

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56

C2949-PCT.txt

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<211> 34

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<223> scFv-KRIX-1VH reverse primer

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<223> scFv-KRIX-1VLVH with His(6)tag forward primer

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<223> scFv-KRIX-1VLVH with His(6)tag reverse primer

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46

<210> 24

<211> 46

<212> DNA

<213> Artificial Sequence

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<223> scFv- Asn47Gln KRIX-1VLVH(His) reverse primer

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46

<210> 25

<211> 867

<212> DNA

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<222> (1)..(867)

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Met	Glu	Thr	Pro	Ala	Gln	Leu	Leu	Phe	Leu	Leu	Leu	Leu	Trp	Leu	Pro	
1				5				10					15			

gat	acc	acc	gga	gaa	att	gtg	ttg	acg	cag	tct	cca	ggc	acc	ctg	tct	96
Asp	Thr	Thr	Gly	Glu	Ile	Val	Leu	Thr	Gln	Ser	Pro	Gly	Thr	Leu	Ser	
			20				25					30				

ttg	tct	cca	ggg	gaa	aga	gcc	acc	ctc	tcc	tgc	agg	gcc	agt	cag	agt	144
Leu	Ser	Pro	Gly	Glu	Arg	Ala	Thr	Leu	Ser	Cys	Arg	Ala	Ser	Gln	Ser	
		35				40					45					

gtt	gcc	agc	gcc	tac	tta	gcc	tgg	tac	cag	caa	aaa	cct	ggc	cag	gct	192
Val	Ala	Ser	Ala	Tyr	Leu	Ala	Trp	Tyr	Gln	Gln	Lys	Pro	Gly	Gln	Ala	
	50					55					60					

ccc	agg	ctc	ctc	atc	tat	ggt	gca	tcc	agt	agg	gcc	acc	gac	atc	cca	240
Pro	Arg	Leu	Leu	Ile	Tyr	Gly	Ala	Ser	Ser	Arg	Ala	Thr	Asp	Ile	Pro	
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C2949-PCT.txt

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				85					90					95		
agc	aga	ctg	gag	cct	gaa	gat	ttt	gca	gtg	tac	tac	tgt	cag	caa	tat	336
Ser	Arg	Leu	Glu	Pro	Glu	Asp	Phe	Ala	Val	Tyr	Tyr	Cys	Gln	Gln	Tyr	
			100					105					110			
ggt	acc	tca	gcc	tta	ctc	act	ttc	ggc	gga	ggg	acc	aag	gtg	gag	atc	384
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		115					120					125				
aaa	cga	ggt	gga	ggc	ggt	tca	ggc	gga	ggt	ggc	tct	ggc	ggt	ggc	gga	432
Lys	Arg	Gly	Gly	Gly	Gly	Ser	Gly	Gly	Gly	Gly	Ser	Gly	Gly	Gly	Gly	
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Ala	Ser	Val	Lys	Val	Ser	Cys	Lys	Thr	Ser	Gly	Tyr	Gln	Phe	Thr	Gly	
				165					170					175		
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Tyr	Ser	Ala	Ser	Gly	His	Ile	Phe	Thr	Ala	Tyr	Ser	Val	His	Trp	Val	
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cga	cag	gcc	cct	gga	caa	ggg	ctt	gag	tgg	atg	gga	agg	atc	aac	cct	624
Arg	Gln	Ala	Pro	Gly	Gln	Gly	Leu	Glu	Trp	Met	Gly	Arg	Ile	Asn	Pro	
		195					200					205				
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Asn	Ser	Gly	Ala	Thr	Asp	Tyr	Ala	His	Lys	Phe	Gln	Gly	Arg	Val	Thr	
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225					230					235					240	
ctg	aca	tct	gac	gac	aca	gcc	atg	tat	tac	tgt	gcg	aga	gcc	gac	aac	768
Leu	Thr	Ser	Asp	Asp	Thr	Ala	Met	Tyr	Tyr	Cys	Ala	Arg	Ala	Asp	Asn	
				245					250					255		
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Tyr	Phe	Asp	Ile	Val	Thr	Gly	Tyr	Thr	Ser	His	Tyr	Phe	Asp	Tyr	Trp	
			260					265					270			
ggc	cgg	gga	acc	ctg	gtc	acc	gtc	tcc	tca	cat	cat	cat	cat	cat	cat	864
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C2949-PCT.txt

<223> scFv- Asn47Gln KRIX-1VLVH(His)

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 20 25 30

Leu Ser Pro Gly Glu Arg Ala Thr Leu Ser Cys Arg Ala Ser Gln Ser
 35 40 45

Val Ala Ser Ala Tyr Leu Ala Trp Tyr Gln Gln Lys Pro Gly Gln Ala
 50 55 60

Pro Arg Leu Leu Ile Tyr Gly Ala Ser Ser Arg Ala Thr Asp Ile Pro
 65 70 75 80

His Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile
 85 90 95

Ser Arg Leu Glu Pro Glu Asp Phe Ala Val Tyr Tyr Cys Gln Gln Tyr
 100 105 110

Gly Thr Ser Ala Leu Leu Thr Phe Gly Gly Gly Thr Lys Val Glu Ile
 115 120 125

Lys Arg Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly
 130 135 140

Ser Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly
 145 150 155 160

Ala Ser Val Lys Val Ser Cys Lys Thr Ser Gly Tyr Gln Phe Thr Gly
 165 170 175

Tyr Ser Ala Ser Gly His Ile Phe Thr Ala Tyr Ser Val His Trp Val
 180 185 190

Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met Gly Arg Ile Asn Pro
 195 200 205

Asn Ser Gly Ala Thr Asp Tyr Ala His Lys Phe Gln Gly Arg Val Thr
 210 215 220

Met Ser Arg Asp Thr Ser Ile Ser Thr Ala Tyr Met Glu Leu Ser Arg
 225 230 235 240

Leu Thr Ser Asp Asp Thr Ala Met Tyr Tyr Cys Ala Arg Ala Asp Asn
 245 250 255

C2949-PCT.txt

Tyr Phe Asp Ile Val Thr Gly Tyr Thr Ser His Tyr Phe Asp Tyr Trp
 260 265 270

Gly Arg Gly Thr Leu Val Thr Val Ser Ser His His His His His His
 275 280 285

<210> 27

<211> 40

<212> DNA

<213> Artificial Sequence

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<223> CHO-scFVKRIX-1VLVHQ(His) forward primer

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40

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<211> 54

<212> DNA

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<223> CHO-scFVKRIX-1VLVHQ(His) reverse primer

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ccggaattct caatgatgat gatgatgatg tgaggagacg gtgaccaggg ttcc

54

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<212> DNA

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<222> (352)..(384)

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1 5 10 15		
gtc cag tcc cag gtg cag ctg gtg cag tct ggg gct gag gtg aag aag		96
Val Gln Ser Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys		
20 25 30		
ccc ggg tcg tcg gtg atg gtc tcc tgc aag gct tct gga ggc acc ttc		144
Pro Gly Ser Ser Val Met Val Ser Cys Lys Ala Ser Gly Gly Thr Phe		
35 40 45		
agc agc ttt ggt atc agc tgg gtg cga cag gcc cct gga caa ggg ctt		192
Ser Ser Phe Gly Ile Ser Trp Val Arg Gln Ala Pro Gly Gln Gly Leu		
50 55 60		
gag tgg gtg gga ggg atc atc cct atc ttt ggt aca gca aac acc gca		240
Glu Trp Val Gly Gly Ile Ile Pro Ile Phe Gly Thr Ala Asn Thr Ala		
65 70 75 80		
cgg aac ttc cag aat aga gtc acc att acc gcg gac gaa ttc acg agc		288
Arg Asn Phe Gln Asn Arg Val Thr Ile Thr Ala Asp Glu Phe Thr Ser		
85 90 95		
aca gcc tac ata cga ctg agg agc ctg aga tct gaa gat acg gcc gtg		336
Thr Ala Tyr Ile Arg Leu Arg Ser Leu Arg Ser Glu Asp Thr Ala Val		
100 105 110		
tat tac tgt gtc ggc ggt cga gat gcc tac agc ttt gat ggt ttt gat		384
Tyr Tyr Cys Val Gly Gly Arg Asp Ala Tyr Ser Phe Asp Gly Phe Asp		
115 120 125		

C2949-PCT.txt

gtc tgg ggc caa ggg aca atg gtc acc gtc tct tca gcc tcc acc aag 432
 Val Trp Gly Gln Gly Thr Met Val Thr Val Ser Ser Ala Ser Thr Lys
 130 135 140

ggc cca tcg gtc ttc ccc 450
 Gly Pro Ser Val Phe Pro
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Val Gln Ser Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys
 20 25 30

Pro Gly Ser Ser Val Met Val Ser Cys Lys Ala Ser Gly Gly Thr Phe
 35 40 45

Ser Ser Phe Gly Ile Ser Trp Val Arg Gln Ala Pro Gly Gln Gly Leu
 50 55 60

C2949-PCT.txt

Glu Trp Val Gly Gly Ile Ile Pro Ile Phe Gly Thr Ala Asn Thr Ala
65 70 75 80

Arg Asn Phe Gln Asn Arg Val Thr Ile Thr Ala Asp Glu Phe Thr Ser
85 90 95

Thr Ala Tyr Ile Arg Leu Arg Ser Leu Arg Ser Glu Asp Thr Ala Val
100 105 110

Tyr Tyr Cys Val Gly Gly Arg Asp Ala Tyr Ser Phe Asp Gly Phe Asp
115 120 125

Val Trp Gly Gln Gly Thr Met Val Thr Val Ser Ser Ala Ser Thr Lys
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Gly Pro Ser Val Phe Pro
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Met	Ala	Trp	Ile	Pro	Leu	Phe	Leu	Gly	Val	Leu	Val	Tyr	Cys	Thr	Gly	
1				5					10					15		

tcc	gtg	gcc	tcc	tct	ggg	ctg	act	cag	cca	cac	tca	gtg	tcc	gtg	tcc	96
Ser	Val	Ala	Ser	Ser	Gly	Leu	Thr	Gln	Pro	His	Ser	Val	Ser	Val	Ser	
			20					25					30			

cca	gga	cag	aca	gcc	aac	atc	acc	tgc	tct	aga	gat	aag	ttg	ggt	cat	144
Pro	Gly	Gln	Thr	Ala	Asn	Ile	Thr	Cys	Ser	Arg	Asp	Lys	Leu	Gly	His	
		35					40					45				

aaa	ttt	gct	tcc	tgg	tat	caa	cag	aag	cca	ggc	cag	tcc	cct	gct	ctt	192
Lys	Phe	Ala	Ser	Trp	Tyr	Gln	Gln	Lys	Pro	Gly	Gln	Ser	Pro	Ala	Leu	
	50					55					60					

ctc	atc	tat	caa	gac	agc	aag	cgg	ccc	tca	ggg	atc	cct	gag	cga	ttc	240
Leu	Ile	Tyr	Gln	Asp	Ser	Lys	Arg	Pro	Ser	Gly	Ile	Pro	Glu	Arg	Phe	
65				70						75					80	

tct	ggc	tcc	aac	tct	ggg	aac	aca	gcc	act	ctg	acc	atc	agc	ggg	acc	288
Ser	Gly	Ser	Asn	Ser	Gly	Asn	Thr	Ala	Thr	Leu	Thr	Ile	Ser	Gly	Thr	
			85					90						95		

cag	gct	atg	gat	gag	gct	gac	tat	tac	tgt	cag	gcg	tgg	gac	aac	acc	336
Gln	Ala	Met	Asp	Glu	Ala	Asp	Tyr	Tyr	Cys	Gln	Ala	Trp	Asp	Asn	Thr	
			100					105					110			

act	gcc	gta	ttc	ggc	gga	ggg	acc	aag	ttg	aca	gtc	cta	agt	cag	ccc	384
Thr	Ala	Val	Phe	Gly	Gly	Gly	Thr	Lys	Leu	Thr	Val	Leu	Ser	Gln	Pro	
		115					120					125				

aag	gct	gcc	ccc	tcg	gtc	act	ctg	ttc	ccg	ccc	tcc					420
Lys	Ala	Ala	Pro	Ser	Val	Thr	Leu	Phe	Pro	Pro	Ser					
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Met Ala Trp Ile Pro Leu Phe Leu Gly Val Leu Val Tyr Cys Thr Gly
 1 5 10 15

Ser Val Ala Ser Ser Gly Leu Thr Gln Pro His Ser Val Ser Val Ser
 20 25 30

Pro Gly Gln Thr Ala Asn Ile Thr Cys Ser Arg Asp Lys Leu Gly His
 35 40 45

Lys Phe Ala Ser Trp Tyr Gln Gln Lys Pro Gly Gln Ser Pro Ala Leu
 50 55 60

Leu Ile Tyr Gln Asp Ser Lys Arg Pro Ser Gly Ile Pro Glu Arg Phe
 65 70 75 80

Ser Gly Ser Asn Ser Gly Asn Thr Ala Thr Leu Thr Ile Ser Gly Thr
 85 90 95

Gln Ala Met Asp Glu Ala Asp Tyr Tyr Cys Gln Ala Trp Asp Asn Thr
 100 105 110

Thr Ala Val Phe Gly Gly Gly Thr Lys Leu Thr Val Leu Ser Gln Pro
 115 120 125

Lys Ala Ala Pro Ser Val Thr Leu Phe Pro Pro Ser
 130 135 140